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BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

VOL. XXX JUNE, 1916 No. 6

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All communications and manuscripts should be sent to the Managing Editor. the University of Chicago, Sept. 15th to June 15th, or Woods Hole, Mass., June 15th to Sept. 15th. Subscriptions and other matter should be addressed to the Biological Bulletin, 41 North Queen Street, Lancaster, Pa

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BIOLOGICAL BULLETIN

THE MARINE BIOLOGICAL LABORATORY

EIGHTEENTH REPORT; FOR THE YEAR 1915

TWENTY-EIGHTH YEAR.

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I. TRUSTEES

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TO SERVE UNTIL 1915

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R. A. HARPER.....Columbia University.
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JACQUES LOEB.....The Rockefeller Institute for Medical Research.
F. P. MALL.....Johns Hopkins University.
GEORGE T. MOORE.....Missouri Botanical Garden, St. Louis.
L. L. NUNN.....Telluride, Colo.
JOHN C. PHILLIPS.....299 Berkeley Street, Boston, Mass.

II. ACT OF INCORPORATION

No. 3170.

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns,

Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our LORD ONE THOUSAND, EIGHT HUNDRED and EIGHTY-EIGHT.

HENRY B. PIERCE,
Secretary of the Commonwealth.

[SEAL.]

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 12 o'clock noon, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk, who shall be, *ex officio*, members of the Board of Trustees, and Tustees as hereinafter provided. At the annual meeting to be held in 1897, not more than twenty-four Trustees shall be chosen, who shall be divided into four classes, to serve one, two, three, and four years, respectively, and thereafter not more than eight Trustees shall be chosen annually for the term of four years. These officers shall hold their respective offices until others are chosen and qualified in their stead. The Director and Assistant Director, who shall be chosen by the Trustees, shall also be Trustees, *ex officio*.

II. Special meetings of the members may be called by the Trustees, to be held in Boston or in Woods Hole at such time and place as may be designated.

III. The Clerk shall give notice of meetings of the members by publication in some daily newspaper published in Boston at least fifteen days before such meeting, and in case of a special meeting the notice shall state the purpose for which it is called.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President and may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The President shall annually appoint two Trustees, who shall constitute a committee on finance, to examine from time to time the books and accounts of the Treasurer, and to audit his accounts at the close of the year. No investments of the funds of the Corporation shall be made by the Treasurer except approved by the finance committee in writing.

VIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be given to the Boston Society of Natural History, or some similar public institution, on such terms as may then be agreed upon.

IX. These By-Laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-Laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. TREASURER'S REPORT

Inasmuch as the records of the Laboratory have been reorganized on an income and expense basis, taking into account the bills receivable and payable, as well as the cash receipts and disbursements, the treasurer makes a departure from the form of report previously employed in order to be in accord with the new methods.

The cash statement of operations is, therefore, now presented in consolidated form and the income and expense schedule follows accompanied by a balance-sheet, showing the condition of the Laboratory on December 31, 1915, as far as the figures are available. Figures for the plant and inventory valuations are in process of compilation.

CONSOLIDATED CASH STATEMENT OF RECEIPTS AND DISBURSEMENTS FOR THE YEAR ENDED DECEMBER 31, 1915

OPERATING ACCOUNTS

Cash on hand January 1, 1915			$1,686.68
Receipts from departments		$46,676.99	
Receipts from donations		35,500.00	
Receipts from work done for others		1,402.16	
Receipts from loans repaid		125.00	
Total receipts for year		$83,704.15	
Payments for departmental expenses	$61,856.38		
Payments for improvements	16,863.51		
Payments for work done for others	1,537.05		
Payments for new town landing	761.50		
Payments for Loan	25.00		
Total payments		81,043.44	
Excess of receipts for year			2,660.71
			$4,347.39
Less Oklahoma warrant included in cash receipts but not yet paid			135.23
Cash balance December 31, 1915			$4,212.16

Cash Receipts and Payments on Account of Investments For the Year ended December 31, 1915

Reserve Fund

Cash on hand January 1, 1915	$210.30	
Receipts:		
Interest —$3,000 American Telephone & Telegraph Company, 4 per cent bonds	60.00	
¾ of $500 Western Telephone & Telegraph Company 5's	18.74	
Dividend—6 shares American Smelting & Refining Company, preferred	42.00	
8 shares General Electric Company	64.00	
14 shares United Shoe Machinery Corporation, preferred	21.00	
2 shares Massachusetts Gas, preferred	8.00	
Interest on bank deposit	1.04	
	$425.08	
Payments:		
Purchase of 2 shares Massachusetts Gas Companies, preferred	180.25	$244.83

Lucretia Crocker Fund

Cash on hand January 1, 1915	$ 99.04	
Receipts:		
Interest —1/5 of $1,000 American Telephone & Telegraph Company 4 per cent. bond	8.00	
Dividend—18 shares Vermont & Massachusetts Railroad Company	108.00	
1 share West End Street Railway Company	3.50	
1 share American Telephone & Telegraph Company	8.00	
2½ shares General Electric Company	20.00	
	$246.54	
Payments:		
2 Scholarships	100.00	146.54

LIBRARY FUND

Cash on hand January 1, 1915	$174.16	
Receipts:		
Interest —4/5 of $1,000 American Telephone & Telegraph Company 4's	32.00	
¼ of $500 Western Telephone & & Telegraph Company, 5's	6.26	
Dividends—3 shares American Telephone & Telegraph Company	24.00	
1 share American Smelting & Refining Company, preferred	7.00	
2½ shares General Electric Company	20.00	
5 shares United Shoe Machinery Company, preferred	7.51	
2 shares Massachusetts Gas Companies, preferred	8.00	
	$278.93	
Payments:		
Purchase 2 shares Massachusetts Gas Companies, preferred	181.26	97.67
Cash on hand December 31, 1915		$489.04

INCOME AND EXPENSE FOR YEAR ENDED DECEMBER 31, 1915

	Expense	*Income*
Administration expense	$ 6,805.36	
BIOLOGICAL BULLETIN	2,594.69	$ 1,523.36
Boat department expense	6,629.63	
Carpenter department expense	983.09	
Chemical department expense	1,430.82	
Dormitories	1,489.49	1,691.04
Fish trap	1,159.21	667.21
Instruction	3,435.98	5,150.00
Philosophical lectures	100.00	
Library department expense	2,628.64	
Mess	15,753.01	16,165.48
Membership dues		1,000.00

Maintenance, buildings and grounds....	5,220.23	
New laboratory expense.....	1,927.76	
Pumping station expense.....	464.70	
Research income.....		3,175.00
Sundry expense and income.....	2,165.48	3,255.25
Supply department.....	12,822.40	19,150.77
Interest on notes payable.....	150.00	
Total expense.....	$65,760.49	
Total income.....	51,778.11	$51,778.11
Excess of expense.....	$13,982.38	
Contribution by Mr. C. R. Crane.....	20,000.00	
Excess carried to balancing account.....	$ 6,017.62	

BALANCE-SHEET, DECEMBER 31, 1915

Assets		*Liabilities and Capital*	
Cash, bank.....	$ 4,212.16	Accounts payable.	$ 1,394.02
Petty cash.....	200.00	Note payable....	3,000.00
Accounts receivable	7,642.37	Trust funds.....	8,222.55
Investments.....	10,733.51	Balancing account	33,997.98
Investment cash...	489.04		
Inventories.....			
Plant account.....			
Improvements, 1915	23,337.47		
	$46,614.55		$46,614.55

D. BLAKELY HOAR, ESQ., Treasurer,
Marine Biological Laboratory
Woods Hole, Mass.
161 Devonshire Street, Boston.

Dear Sir: We have audited the accounts of the Marine Biological Laboratory as kept at Woods Hole and of the trust funds and accounts as kept at your office, 161 Devonshire Street, for the year ended December 31, 1915.

We have checked the report of the Treasurer submitted above and find it correct and in accord with the books. The extent of the audit together with the supporting schedule is set forth in a detailed report under date of February 1, 1916.

Very respectfully,

HARVEY S. CHASE & CO.,
Certified Public Accountants

V. LIBRARIAN'S REPORT

AUGUST, 1915

Since the last annual report the reorganization of the library has been carried on by Miss Scott, who has brought each department up to a state of system and efficiency.

We can now make a more definite statement of the contents of the library, including a number of accessions not previously listed: Total number of accessions 10,046. Old and new accessions recorded since last report 6,746. Of these there have been added since 1914:

934 volumes loaned by the American Museum of Natural History,
466 volumes given by the Wisconsin Academy of Sciences,
200 " given by Mr. Crane,
720 " bound during year.

These volumes may be conveniently divided as follows:

Journals, for which most of our appropriation is expended and which form the bulk of the library, in bound sets	95
Serials and publications of academies, museums, laboratories, and societies (many of these are from exchanges which are steadily increasing)	188
Books:	
Zoology	321
Botany	134
Physiology	100
Hygiene and medicine	74
Chemistry	18
Evolution	82
Psychology	36
Philosophy	104
Miscellaneous	198
Total	1,067

We can buy very few books and are dependent upon gifts from authors and publishers, who recognize that this is a particularly useful place to have their works examined.

The separates number 4,000. Our reprint collection might easily be notably increased through the aid of authors and friends. Such duplicates are in great demand.

The following advances have been made. First, and very important, is the considerable number of missing parts which

have been secured by Miss Scott's enterprise toward completing back volumes of sets.

These have been completed: *Nature, Wilson Bulletin, University of California Publications in Zoölogy, Colorado University Studies, Bergens Museum Publications.*

These have been added to but are still incomplete:

Annales d. Sciences Naturelles—Zoölogy,
Bulletin Museum of Comparative Zoölogy, Harvard,
Proceedings Boston Society of Natural History,
Proceedings American Philosophical Society,
American Naturalist,
American Journal of Science and Arts,
Journal Medical Research,
Field Columbian Museum Publications,
Bureau of Fisheries, Bulletin and Report,
Contributions U. S. National Herbarium,
Proceedings American Association for the Advancement of Science,
Proceedings Iowa Academy of Sciences,
Proceedings Indiana Academy of Sciences.

The following new sets have been added to the library:

Transactions American Microscopical Society,
Transactions Wisconsin Academy of Sciences,
Bulletin Torrey Botanical Club,
American Journal of Botany,
Proceedings Academy of Natural Sciences, Philadelphia,
Journal of Parasitology,
Illinois Biological Monographs,
Unpopular Review,
Annals Missouri Botanical Garden,
Indiana University Studies,
U. S. Bureau of Education, Report and Bulletin,
U. S. War Department, Bulletin,
Maine Agricultural Experiment Station, Report and Bulletin,
Museum of Brooklyn Institute of Arts and Sciences, Science Bulletin.

Seventy-six volumes of Hoppe-Seyler's *Zeitschrift für Physiologische Chemie* were purchased from Miss Koch for $200, and

the remainder of the set, which is now in the 94th volume, has been secured.

Exchanges now number 29; six have been arranged this year: *Indian Museum; American Journal of Botany; New York Academy of Sciences; Academy of Natural Sciences, Philadelphia; Biologische Untersuchungen, Stockholm;* and *Zoologiska Bidrag*, Upsala. New subscriptions have been placed for the *Popular Science Monthly* and the *English Journal of Physiology.*

Here we must report a partial disposition of the Journal Fund which was begun in 1912. Since then, a number have subscribed to this fund which is to enable us to secure new files of journals. Drs. Mayer, Knower, Meigs, Rice and Just have subscribed from $5 to $10 a year for a period of five years. This has already furnished $55 and will still accumulate from new gifts.

In addition, last year Mr. Tashiro and a number of other investigators, interested in securing the *English Journal of Physiology*, subscribed amounts from 50 cents to $1 each, thus securing $7.50. This sum has now been increased to $12. For this, we must thank Drs. Tashiro, Heilbrunn, Gould, Wherry, Packard, Sturtevant, Bridges, Harvey, Metz, and Morgan, and Misses Hoge, Medes, Browne, and Dunn. Such coöperation is most encouraging. It makes possible a purchase long needed. We have subscribed to the *Journal* with this gift and have used the amount already accumulated for the Journal Fund to purchase a number of back volumes, hoping gradually complete to the set.

Further growth in this spirit of coöperation in building up the library is shown in the list of important gifts.

Various publishers have given a total of 16 books this year. Miss Fay has given several quite valuable books on mushrooms. Mr. Crane gave us two hundred volumes from his personal library, as well as two very interesting Russian curios. Dr. George T. Moore gave duplicates from his library, 28 bound volumes and 21 separates. Dr. Philipp Fischelis donated 53 valuable papers by Kowalewsky and other Russian authors. Dr. Conklin has given his book on "Heredity and Environment," Dr. Parker, his book on "Biology and Social Problems," and Dr. Abbott has ordered a copy of his book on "General Biology" sent to us. Dr. Calkins also presented his texbook on "Biology."

The Wistar Institute gave a set of the "Biological Lectures," Mrs. Gardiner donating Volume I. to complete our set. Dr. Ward gave a subscription to the *Journal of Parasitology*, and Dr. Bumpus to the current volume of the *American Naturalist* and the *Unpopular Review*. Dr. Gustav Retzius has promised to send a complete set of his works as soon as transportation is safer. Three hundred and twelve reprints have been presented to the library this year by various authors.

Through the coöperation of Dr. George Wagner, a large number of duplicates from the library of the Wisconsin Academy of Sciences are being transferred to this library; 466 volumes have been received and the number will probably reach 1,000.

The Prince of Monaco has sent us about 75 photographs of the buildings and equipment of the Institut Oceanographique, together with pamphlets telling about the foundation and progress of the institute. A number of other stations have sent various literature and photographs, and it is hoped to secure material of this kind from all biological stations in the world.

We wish to acknowledge the coöperation of Drs. Moore and Duggar in securing the *Bulletin of the Torrey Botanical Club*, and of Dr. Ivey Lewis in arranging and preparing for the binder a most confusing set of plates.

We are really in need of a number of journals that we do not have, and we should complete as soon as possible certain sets that have long been defective. The library needs an increased appropriation for handbooks, monographs, and general works like the Encyclopedia Britannica, the Century Dictionary, etc.

Above all, we must point out the great value of reprints and books sent here by individuals, and the importance of personal efforts in behalf of the library on the part of the biologists who use it. Such interest has already done much to build it up.

VI. THE DIRECTOR'S REPORT

To the Trustees of the Marine Biological Laboratory:

Gentlemen: The season of 1915, the twenty-eighth session of the Marine Biological Laboratory, was marked by a farther

advance in many of the statistics of the Laboratory as will appear from the appended lists (1) of the Staff, (2) of the Investigators and Students, (3) the Tabular View of Attendance, (4) the Subscribing Institutions, (5) of the Evening Lectures, (6) of the Members of the Corporation. The Treasurer's report and the Librarian's report also show a healthy condition of growth. We have much cause for congratulation, and reason for deep thankfulness to Mr. Crane without whose whole-hearted financial support and intelligent sympathy with our objects, our best efforts must have been comparatively ineffective.

The number of investigators in attendance was 137 as compared with 129 in 1914, 122 in 1913 and 93 in 1912; the number of students was 105 as compared with 89 in 1914, 69 in 1913 and 67 in 1912. The total attendance was 242 as compared with 218 in 1914, 191 in 1913 and 160 in 1912. These figures bear testimony to the growing appreciation of the facilities furnished by the Laboratory both in research and in instruction. The number of institutions represented by these workers was 79, which is almost the same as in the two preceding years; the increase was therefore due to larger attendance from certain institutions. Indeed no considerable increase in the number of institutions represented is to be expected, because practically all of the larger institutions of higher education in the East and Middle West are represented each year, and the variations from year to year are accounted for by fluctuations of a more or less fortuitous character among the smaller institutions represented.

The receipts from subscribing institutions and fees were $8,325 as compared with $7,300 in 1914, $6,160 in 1913 and $5,175 in 1912. The Supply Department has also shown an encouraging increase, having filled 282 more orders than in 1914 with total paid receipts of $16,932.00 as compared with $14,003.35 in 1914.

Three important additions to the property and equipment of the Laboratory were made during the year: (1) The so-called Bake House property adjoining the Laboratory property on the east was purchased; this piece has about 103 feet frontage on the Eel pond and on the main street by 100 feet in depth, and its title carries with it the private roadway previously controlled jointly by the Laboratory and the owners of this property. The

old house on the property has been fitted up as a workshop for the carpenters' and plumbers' department, which was very badly needed. The frontage on the eel pond is especially valuable for future development. (2) The Laboratory also purchased the grounds and house known as the Ritter property, 78½ by 100 feet, adjacent to the Whitman house, and opposite the lecture hall. The house furnished much needed enlargement of our dormitory facilities. (3) The old "Homestead" hitherto used as the matron's and helpers' house, adjoining the mess, was torn down and replaced by a much larger, modern and attractive dwelling house with accommodations for 43 persons. As all of this space was not needed for the mess, part of it was used for a woman's dormitory. We owe these three splendid additions to the facilities of the Laboratory, as I need hardly say, to the continued generosity, and thoughtfulness in the matter of all Laboratory needs, of the President of the Board of Trustees, Mr. Crane. Other improvements during the year were as follows: The steamer *Cayadetta* was thoroughly overhauled and her deck raised 18 inches at a cost of over $2,000, so that she is a much stauncher and more seaworthy boat than ever before. The filling in of the Laboratory harbor frontage, including the Yacht Club, has been completed; this will be graded and planted in the spring and the building moved to the east end of the frontage away from the center of the new laboratory building. Very considerable additions were made to the kitchen and laundry of the mess, power machinery electrically operated being installed for all the major operations, such as dish-washing, washing and drying of laundry, freezing ice-cream, etc. The work has thus been greatly lightened and facilitated. The Laboratory also transferred a 40-foot strip at the extreme north and west end of our Eel pond frontage to the town of Falmouth for the purposes of a boat landing, which has now been built at a cost of about $1,500, equally divided between the town and Laboratory. This was in pursuance of an agreement entered into with the town at the time of the construction of the drawbridge over the Eel pond entrance. It is therefore now possible to use the Eel pond as a harbor, and to secure delivery of supplies by this back entrance to the Laboratory property.

With all of the enlargements and additions of the past few years the Laboratory is still badly crowded during the height of the season. This applies to a certain extent to the actual accommodations for workers in the laboratory buildings, but more particularly to the housing accommodations in the village. We are planning to meet the former condition by restricting numbers in the larger classes; applications for admission will be received up to May, and appointments then made in accordance with the number of working places; if places are still available after such assignments later applicants can be admitted. It is probable that by means of various adjustments we can continue to care for all qualified investigators.

The housing accommodations in the village, however, raise a more serious question. They are entirely inadequate, and there is a consequent tendency for the prices of rooms to advance, which results in discouraging attendance. Investigators who wish to carry on regular work at the Laboratory are unable to rent houses at reasonable rates for the accommodation of their families. Conditions have become increasingly discouraging in these respects for the past several years. Moreover, land is almost unavailable for those families who wish to solve the living problem by building. It is undoubted that these conditions will exercise an inhibiting influence on the work of the Laboratory in the future, and one of the most important problems before us is to devise some means of counteracting them.

As an organization we have laid the principle of coöperation at our foundation, and we have attempted to build it into every one of our activities. Our Board of Trustees is representative of the institutions of learning of the country; our corporation represents the workers in biology on the broadest lines we can secure; our staff of instructors is widely drawn from different institutions; our students and investigators come from all parts of the accessible territory, and some from abroad; we ask only that they have a fit preparation and exhibit the spirit of scholarship. We are not tied down to any one institution or small section of the country. In past years we have considered these principles worth struggling for, and we have more than once sacrificed security to freedom of action. Our recent years have been free

and comfortable. We must not allow ourselves to forget that the principles for which we stand are never entirely won; and I appeal therefore to every member of the Board of Trustees and to all members of the corporation not to allow a sense of security to dull the edge of devotion, to keep the interests of the Laboratory at heart and to work for their support in all possible ways.

I. THE STAFF

1915

FRANK R. LILLIE, DIRECTOR,
Professor of Embryology, and Chairman of the Department of Zoölogy, The University of Chicago.

GILMAN A. DREW, ASSISTANT DIRECTOR,
Marine Biological Laboratory.

ZOÖLOGY

I. INVESTIGATION

GARY N. CALKINS.....Professor of Protozoölogy, Columbia University.
E. G. CONKLIN.....Professor of Zoölogy, Princeton University.
GILMAN A. DREW.....Assistant Director, Marine Biological Laboratory.
GEORGE LEFEVRE.....Professor of Zoölogy, The University of Missouri.
FRANK R. LILLIE.....Professor of Embryology, The University of Chicago.
C. E. MCCLUNG.....Professor of Zoölogy, University of Pennsylvania.
T. H. MORGAN.....Professor of Experimental Zoölogy, Columbia University.
E. B. WILSON.....Professor of Zoölogy, Columbia University.

II. INSTRUCTION

CASWELL GRAVE.....Associate Professor of Zoölogy, Johns Hopkins University.
W. C. ALLEE.....Assistant Professor of Zoölogy, University of Oklahoma.

GEORGE A. BAITSELL.....Instructor in Biology, Yale University.
RAYMOND BINFORD.....Professor of Biology, Earlham College.
E. J. LUND.....Instructor in Protozoölogy, University of Pennsylvania.
T. S. PAINTER.....Instructor in Biology, Yale University.

EMBRYOLOGY

I. INVESTIGATION (see Zoölogy)

II. INSTRUCTION

WILLIAM E. KELLICOTT...Professor of Biology, Goucher College.
ROBERT A. BUDINGTON....Professor of Zoölogy, Oberlin College. (Absent in 1915.)
J. F. ABBOTT.....Professor of Zoölogy, Washington University.
CHARLES PACKARD.....Instructor in Zoölogy, Columbia University.
CHARLES C. ROGERS.....Professor of Zoölogy, Oberlin College.

PHYSIOLOGY

I. INVESTIGATION

ALBERT P. MATHEWS.....Professor of Physiological Chemistry, The University of Chicago.
RALPH S. LILLIE.....Professor of Biology, Clark University.
HAROLD C. BRADLEY...Assistant Professor of Physiological Chemistry, University of Wisconsin.
SHIRO TASHIRO.....Instructor in Physiological Chemistry, The University of Chicago.

II. INSTRUCTION

RALPH S. LILLIE.....Professor of Biology, Clark University.
WALTER E. GARREY.....Associate Professor of Physiology, Washington University Medical School.
FRANK P. KNOWLTON.....Professor of Physiology, Syracuse University.
EDWARD B. MEIGS.....Associate in Physiology, Wistar Institute of Anatomy and Biology.

PHILOSOPHICAL ASPECTS OF BIOLOGY AND ALLIED SCIENCES

LECTURES

EDWARD G. SPAULDING....Professor of Philosophy, Princeton University.

BOTANY

GEORGE T. MOORE.....Director, Missouri Botanical Garden and Professor of Botany, Washington University.

B. M. DUGGAR.....Physiologist, Missouri Botanical Garden and Professor of Plant Physiology, Washington University. (Absent in 1915.)

IVEY F. LEWIS.....Professor of Botany, University of Missouri.

R. H. COLLEY.....Instructor in Botany, Dartmouth College.

A. R. DAVIS.....Lackland Research Fellow, Missouri Botanical Garden.

W. H. WESTON.....Graduate Student, Harvard University.

LIBRARY

H. McE. KNOWER.....Professor of Anatomy, University of Cincinnati, Librarian.

MARY E. SCOTT.....Assistant Librarian.

Chemical Supplies

OLIVER S. STRONG.....Instructor in Anatomy, College of Physicians and Surgeons, New York City, Chemist.

Supply Department

G. M. GRAY.....Curator.

JOHN J. VEEDER.....Captain.

E. M. LEWIS.....Engineer.

A. W. LEATHERS.....Collector.

A. M. HILTON.....Collector.

F. G. GUSTAFSON.....Collector in Botany.

EDNA E. WELLS.....Clerk.

F. M. MACNAUGHT.....Business Assistant.

HERBERT A. HILTON.....Superintendent of Buildings and Grounds.

2. INVESTIGATORS AND STUDENTS

1915

A. ZOÖLOGY

Independent Investigators

ABBOTT, JAMES F., Professor of Zoölogy, Washington University.
ADDISON, WILLIAM H. F., Assistant Professor of Normal Histology and Embryology, University of Pennsylvania.
ALLEE, WARDER C., Professor of Biology, Lake Forest College.
ALLEN, EZRA, Professor of Biology, Philadelphia School of Pedagogy.
BAITSELL, GEORGE A., Instructor in Biology, Yale University.
BECKWITH, CORA J., Associate Professor of Zoölogy, Vassar College.
BINFORD, RAYMOND, Professor of Zoölogy, Earlham College, Richmond.
BORING, ALICE M., Associate Professor of Zoölogy, University of Maine.
BROWNE, ETHEL N., 510 Park Ave., Baltimore, Md.
CALKINS, GARY N., Professor of Protozoölogy, Columbia University.
CASTEEL, DANA B., Associate Professor of Zoölogy, University of Texas.
CHIDESTER, FLOYD E., Associate Professor of Zoölogy, Rutgers College.
CHILD, C. M., Associate Professor of Zoölogy, University of Chicago.
CLAPP, CORNELIA M., Professor of Zoölogy, Mt. Holyoke College.
CLARK, ELEANOR L., Columbia, Mo.
CLARK, ELIOT R., Professor of Anatomy, University of Missouri.
CONKLIN, EDWIN G., Professor of Biology, Princeton University.
COPELAND, MANTON, Professor of Biology, Bowdoin College.
COWDRY, E. V., Associate in Anatomy, Johns Hopkins Medical School.
COWDRY, N. H., Johns Hopkins Medical School, Baltimore, Md.
CRAMPTON, H. E., Professor of Zoölogy, Barnard College, Columbia Univ.
DANCHAKOFF, WERA, Woods Hole, Mass.
DEXTER, JOHN S., Professor of Biology, University of Saskatchewan.
DONALDSON, H. H., Wistar Institute of Anatomy and Biology.
DREW, GILMAN A., Assistant Director, Marine Biological Laboratory.
DUNN, ELIZABETH H., Woods Hole, Mass.
GEE, WILSON, Professor of Biology, Emory University, Oxford, Ga.
GOLDFARB, A. J., Professor of Biology, College of the City of New York.
GOLDSCHMIDT, RICHARD, Member of Kaiser Wilhelm Institut Für Biologie.
GRAVE, CASWELL, Associate Professor of Zoölogy, Johns Hopkins University.
GREGORY, EMILY R., Professor of Biology, University of Akron.
GREGORY, LOUISE H., Instructor in Zoölogy, Barnard College.
GROSS, ALFRED O., Bowdoin College, Brunswick, Maine.
HARMAN, MARY T., Assistant Professor of Zoölogy, Kansas State Agricultural College.
HARVEY, BASIL C. H., Associate Professor of Anatomy, University of Chicago.
HEILBRUNN, LEWIS V., Associate in Embryology, University of Chicago.
HEGNER, ROBERT W., Assistant Professor of Zoölogy, University of Michigan.
HOGUE, MARY J., Instructor in Zoölogy, Wellesley College.
JACOBS, MERKEL H., Assistant Professor of Zoölogy, University of Pennsylvania.

KELLICOTT, WILLIAM E., Professor of Biology, Goucher College.
KNOWER, HENRY McE., Professor of Anatomy, University of Cincinnati.
LANE, HENRY H., Professor of Zoölogy, State University of Oklahoma.
LEFEVRE, GEORGE, Professor of Zoölogy, University of Missouri.
LEWIS, MARGARET R., Carnegie Institution.
LEWIS, WARREN H., Professor of Physiological Anatomy, Johns Hopkins Medical School.
LILLIE, FRANK R., Professor of Embryology, University of Chicago.
LUND, ELMER J., Assistant Professor of Zoölogy, University of Minnesota.
MALONE, EDWARD F., Associate Professor of Anatomy, University of Cincinnati.
MACKLIN, CHARLES C., Johns Hopkins Medical School, Baltimore, Md.
MCCLUNG, CLARENCE E., Director of Zoölogical Laboratory, University of Pennsylvania.
MORGAN, T. H., Professor of Experimental Zoölogy, Columbia University.
MORGAN, ANNA H., Associate Professor of Zoölogy, Mt. Holyoke College.
MORRIS, MARGARET, Fellow in Zoölogy, Yale University.
PACKARD, CHARLES, Instructor in Zoölogy, Columbia University.
PAINTER, THEOPHILUS S., Instructor in Biology, Yale University.
PAPPENHEIMER, ALWIN M., Assistant Professor of Pathology, Columbia Univ.
PATTERSON, JOHN T., Professor of Zoölogy, University of Texas.
RICHARDS, A., Instructor in Zoölogy, University of Texas.
ROBERTSON, W. REES B., Assistant Professor of Zoölogy, University of Kansas.
SPAETH, REYNOLD A., Instructor in Biology, Yale University.
SPAULDING, E. G., Professor of Philosophy, Princeton University.
STOCKARD, CHARLES R., Professor of Anatomy, Cornell Medical College.
STREETER, GEORGE L., Research Associate, Carnegie Institution of Washington.
STRONG, OLIVER S., Instructor in Anatomy, Columbia University.
STURTEVANT, ALFRED H., Columbia University, New York City.
TENNENT, DAVID H., Professor of Biology, Bryn Mawr College.
WELCH, PAUL S., Assistant Professor of Entomology, Kansas State Agricultural College.
WOODWARD, ALVALYN E., Fellow in Zoölogy, University of Michigan.
ZELENY, CHARLES, Associate Professor of Zoölogy, University of Illinois.

Beginning Investigators

ADKINS, W. S., Graduate Student, Columbia University.
ALTENBURG, EDGAR, Assistant in Botany, Columbia University.
BINKLEY, LELIA T., University of Texas.
BRIDGES, CALVIN B., Columbia University.
COHN, EDWIN J., University of Chicago.
COLLETT, MARY E., Instructor in Biology, Carnegie Institution of Technology.
FISH, J. Burton, Teacher of Biology, Boys' High School, Brooklyn.
FREIBERG, HENRY B., University of Cincinnati.
GLOBUS, JOSEPH H., Assistant in Anatomy, Cornell Medical College.
GOLDMAN, AGNES, Cornell University.
GOODRICH, H. B., Union College, Schnectady.
GOULD, HARLEY N., Fellow in Biology, Princeton University.
HANCE, ROBERT T., Fellow in Zoölogy, University of Pennsylvania.
HASTINGS, GEORGE T., Teacher of Biology, DeWitt Clinton High School, Yonkers, N. Y.

HAYDEN, MARGARET A., Instructor in Biology, Carnegie Institute of Technology.
HOLT, CAROLINE M., Graduate Student, University of Pennsylvania.
HICKERNELL, LOUIS M., Instructor in Zoölogy, Syracuse University.
HOY, WILLIAM E., JR., Research Student, Princeton University.
JOHNSON, SYDNEY E., Fellow in Zoölogy, Northwestern University.
LYNCH, CLARA J., Instructor, Smith College.
MARKS, BERNICE, 4 East 94th St., New York City.
MEDES, GRACE, Graduate Student, Bryn Mawr College.
MINOURA, TADACHIKA, Zoölogy Department, University of Chicago.
MOORE, CARL R., University of Chicago.
OSTERUD, HJALMAR L., Instructor in Zoölogy, University of Washington
PHILLIPS, RUTH L., Associate Professor of Biology, Western College.
REAGAN, FRANKLIN T., Fellow in Biology, Princeton University.
ROOT, FRANCIS M., Johns Hopkins University.
SAFIR, SHELLEY R., Teacher in New York High School, New York City.
VANNEMAN, AIMÉE S., Technician, University of Texas.
WARE, CLARA C., Graduate Student, Columbia University.
WEINSTEIN, ALEXANDER, Columbia University.
WHEAT, FRANK M., Columbia University.
WHITING, PHINEAS W., University of Pennsylvania.
WHITE, EDITH G., Columbia University.
WILKINS, LAWSON, Johns Hopkins University.

B. PHYSIOLOGY

Independent Investigators

BOGACKI, KAMIL J., Physician, Western Reserve University.
CHAMBERS, ROBERT, JR., Assistant Professor of Histology and Comparative Anatomy, University of Cincinnati.
EDWARDS, DAYTON J., Instructor in Physiology, College of the City of New York.
GARREY, WALTER E., Associate Professor of Physiology, Washington University.
HYDE, IDA H., Professor of Physiology, University of Kansas.
JUST, ERNEST E., Professor of Physiology, Howard University.
KINGSBURY, FRANCIS B., Instructor in Physiological Chemistry, University of Minnesota.
KNOWLTON, FRANK P., Professor of Physiology, Syracuse University.
LILLIE, RALPH S., Professor of Biology, Clark University.
LOEB, JACQUES, Head of Department of Experimental Biology, Rockefeller Institute for Medical Research.
MATHEWS, ALBERT P., Professor of Physiological Chemistry, University of Chicago.
MEIGS, EDWARD B., 1722 H. Street, N. W., Washington, D. C.
MOORE, ARTHUR R., Associate Professor of Physiology, Bryn Mawr College.
ROGERS, CHARLES G., Professor of Comparative Physiology, Oberlin College.
SCOTT, ERNEST L., Associate in Physiology, Columbia University.
TASHIRO, SHIRO, Instructor in Physiological Chemistry, University of Chicago.
WALLER, JOHN C., Graduate Student, University of Chicago.
WARREN, HOWARD C., Professor of Psychology, Princeton University.
WASTENEYS, HARDOLPH, Associate in Experimental Biology, Rockefeller Institute for Medical Research.
WERBER, ERNEST L. Sessel Research Fellow, Yale University.

Beginning Investigators

ATWOOD, W. G., Dartmouth College.
CHAMBERLAIN, MARY M., Bryn Mawr College.
LEVY, AUGUSTUS, University of Chicago.
CATTELL, MCKEEN, Harvard Medical School.

C. BOTANY

Independent Investigators

BLAKESLEE, A. F., Professor of Botany and Genetics, Connecticut Agricultural College.
COLLEY, R. H., Instructor in Botany, Dartmouth College.
HIBBARD, R. PERCIVAL, Plant Physiologist, Michigan Agricultural College.
LEWIS, IVEY F., Professor of Botany, University of Missouri.
MOORE, GEORGE T., Director, Missouri Botanical Garden, St. Louis.
WESTON, WILLIAM H., JR., Instructor in Botany, Harvard University.

Beginning Investigators

COBB, RUTH, Falls Church, Virginia.
SMITH, PEARL M., Assistant in Botany, University of Wisconsin.

STUDENTS

1915

1. ZOÖLOGY

ADAMS, A. ELIZABETH, Instructor in Zoölogy, Mount Holyoke College.
ANDRUS, EDWIN C., Student, Oberlin College, Oberlin, Ohio.
ANDRUS, WILLIAM D. W., Student, Oberlin College, Oberlin, Ohio.
BAIN, THERESE S., Vassar College, Poughkeepsie, N. Y.
BALDWIN, IMOGEN, Student, Mt. Holyoke College, So. Hadley, Mass.
BELL, CHARLES E., Student, Ursinus College.
BLANCHARD, NELLIE P., Head of Biological Dept., Hood College.
CATTELL, OWEN, Garrison-on-Hudson.
CATTELL, PSYCHE, Student, Sargent, Cambridge, Mass.
CHARLTON, HARRY H., Assistant in Zoölogy, Yale University.
COCKS, EDMUND, Graduate Student, Columbia University, New York City.
COBB, MARGARET C., Barnard College, New York City, N. Y.
COWDERY, LAWRENCE T., Student, Oberlin College, Oberlin, Ohio.
CROSS, HOWARD B., Instructor, Oklahoma University, Norman, Okla.
DIBELL, MABEL E., Instructor of Biology, Western College.
EHRENFELD, FREDERICK, Assistant Professor of Geology, University of Pennsylvania.
GILBERT, MARION, Student, Oberlin College, Oberlin, Ohio.
GREISHEIMER, ESTHER M., Clark University, Worcester, Mass.
HARVEY, HELEN F., Oberlin College, Oberlin, Ohio.
HARRIS, COLEMAN J., Lewisburg, Pa.

HAYDEN, RUTH, Goucher College, Baltimore, Md.
HEA, EMILY M., Teacher in High School, Morristown, N. J.
HEEMAN, HARRIET M., Student, Oberlin College, Oberlin, Ohio.
HOWE, MARION G., Mt. Holyoke College, So. Hadley, Mass.
HUGHES, DOROTHEA M., Simmons College, Boston, Mass.
HULST, MYRA M. Vassar College, Poughkeepsie, N. Y.
IRVINE, HELEN, Student, Mt. Holyoke College, So. Hadley, Mass.
JOSEPHS, H. W., Assistant in Chemistry, Harvard University, Cambridge.
KOSTIR, WENCEL J., Instructor, Ohio State University, Columbus, Ohio.
LUDWIG, ALBERT P., Student, Oberlin College, Oberlin, Ohio.
MARINUS, CARLETON J., Syracuse University, Syracuse, N. Y.
MAHR, ERNST F., JR., Syracuse University, Syracuse, N. Y.
MCGRATH, JULE G., Laboratory Assistant, Hunter College, New York City.
MOSES, GERTRUDE, 2002 Bolton St., Baltimore, Md.
OUDESLUYS, HORTENSE, Teacher, Western High School, Baltimore, Md.
PENNYPACKER, FRANCES W., Sweet Briar College.
RIPPLE, CLARENCE V., Student, University of Pennsylvania, Philadelphia.
ROGERS, MILDRED, Student, Goucher College, Baltimore, Md.
RUNYON, PAUL M., Student, Princeton University, Princeton, N. J.
SANFORD, ELDON W., Graduate Student, Yale University, New Haven, Conn.
SHELDON, PAUL B., Student, Oberlin College, Oberlin, Ohio.
SMITH, SUE F., Carnegie Institute of Technology.
SMITH, CHRISTIANNA, Assistant in Zoölogy Laboratory, Mt. Holyoke College, So. Hadley, Mass.
SMITH, INEZ C., Student, Mt. Holyoke College, So. Hadley, Mass.
SPENCE, MARGARET, Instructor La Crosse State Normal School, La Crosse.
WILDER, KATHERINE, Science Assistant, The Newton High School.
WRIGHT, HELEN G., Student, Mt. Holyoke College, So. Hadley, Mass.

2. EMBRYOLOGY

ASHMAN, RICHARD, Rutgers College, New Brunswick, N. J.
ALLARD, ANN D., Teacher, So. Boston, Mass.
BLANCHARD, FRANK H., Instructor, Tufts College, Mass.
BLAU, JULIA E., Princeton, N. J.
CAHN, ALVIN R., Assistant in Zoölogy, University of Wisconsin, Madison, Wis.
CARROLL, MITCHEL, 617 So. 16th St., Philadelphia, Pa.
CLOSSON, JAMES H., Student, Princeton University, Princeton, N. J.
COBB, DOROTHY, Student, Radcliffe College, Cambridge, Mass.
CUTTER, ELIZABETH, Assistant in Zoölogy, Vassar College.
FARNUM, ALICE R., Student, Smith College, Northampton, Mass.
FISH, GORDON T., Assistant, Yale University, New Haven, Conn.
HAMLIN, HOWARD E., Student, Harvard University, Cambridge, Mass.
HENRY, EDNA M., Student, Barnard College, New York City, N. Y.
HERRICK, JOSEPH C., Professor of Biology, St. Joseph's Seminary, Yonkers, N. Y.
HUFFORD, CLARENCE E., Student, Oberlin College, Oberlin, Ohio.
HURLIN, RALPH G., Instructor, Clark College.
JAMIESON, JANET P., Student, University of Pennsylvania.
KAKIUCHI, SAMURO, Yale University, New Haven, Conn.
LEWIS, ELSIE M., Student, Oberlin College, Oberlin, Ohio.

MACY, CORA F., Student, Syracuse University, Syracuse, N. Y.
MALLARD, AGNES K., Teacher, 1658 Columbia Road, So. Boston, Mass.
MYERS, MAE L., Associate Professor of Anatomy, Woman's Medical College of Pennsylvania.
MILLIKAN, FRANCES, Student, Smith College, Northampton, Mass.
MONTGOMERY, PRISCILLA B., 105 So. 41 St., Philadelphia, Pa.
PARMENTER, CHARLES L., Assistant Professor, University of Wisconsin.
PATTEN, MARY W., Goucher College, Baltimore, Md.
RICHARDS, LYMAN G., 259 Prospect St., Fall River, Mass.
RICHARDS, GEORGE L., 124 Franklin St., Fall River, Mass.
SHERWOOD, HELEN L., Student, Vassar College, Poughkeepsie, N. Y.
STOCKING, RUTH J., Professor of Biology, Agnes Scott College, Decatur.
STOCKING, BESSE E., Student, Goucher College, Baltimore, Md.
STRAUS, AUBREY H., Associate Professor of Bacteriology, Medical College of Virginia, Richmond, Va.
THOMAS, ANNA M., Student, Carnegie Institute of Technology.
WEBSTER, LESLIE T., Amherst College, Amherst, Mass.
WILLIAMS, G. HUNTINGTON, Graduate Student, Johns Hopkins Medical School.
WILLIAMS, JAMES W., Lincoln St., New Haven, Conn.
WINSLOW, MINA L., Graduate Student, University of Michigan.

3. PHYSIOLOGY

ADLER, FRANCIS H., University of Pennsylvania, Philadelphia, Pa.
BENSING, LERUE P., Research Assistant, Research Commission of Natural Dental Association, Cleveland, Ohio.
BODINE, JOSEPH H., University of Pennsylvania, Philadelphia, Pa.
FENN, WALLACE O., Harvard University, Cambridge, Mass.
EGGSTEIN, A. A., Instructor in Pathology, Vanderbilt College.
HOLT, EMMETT, 14 West 55th St., New York City, N. Y.
HUGHES, WALTER S., Massachusetts Institute of Technology.
LOEB, ROBERT F., Student, University of Chicago, Chicago, Ill.
LUNDGREN, C. ALBERT, Assistant in Biology, Clark University.
METCALF, JOHN T., Instructor in Psychology, Princeton University.
MINNICH, DWIGHT E., Harvard University, Cambridge, Mass.
PRICE, WESTON A., Chairman, The Research Commission of the National Dental Association, Cleveland, Ohio.
REEVES, PRENTICE, Assistant in Experimental Psychology, Princeton Univ.
SCHNEIDER, PETER A., Instructor in Zoölogy, University of Vermont.
THOMPSON, MARTHA, Teacher of Biology, Bay Ridge High School, New York City, N. Y.

4. BOTANY

MCCONNELL, LOUISE J., 341 Rector St., Perth Amboy, N. J.
MONG, GRACE E., Student, Oberlin College, Oberlin, Ohio.
OAK, DOROTHY, Student, Barnard College, New York City, N. Y.
SEVERY, J. WARREN, Student, Oberlin College, Oberlin, Ohio.
YASUI, KONO, Student, Radcliffe College, Cambridge, Mass.
YOUNG, Anna R., Student, Smith College, Northampton, Mass.

3. TABULAR VIEW OF ATTENDANCE

	1911	1912	1913	1914	1915
INVESTIGATORS—Total	82	93	122	129	137
Independent:					
Zoölogy	42	44	58	62	69
Physiology	18	14	17	22	20
Botany	8	10	11	10	6
Under Instruction:					
Zoölogy	12	21	21	31	36
Physiology	2	2	7	1	4
Botany		2	7	3	2
STUDENTS—Total	65	67	69	89	105
Zoölogy	26	24	33	43	47
Embryology	20	15	22	21	37
Physiology	6	11	8	10	15
Botany	13	17	7	15	6
TOTAL ATTENDANCE	147	160	191	218	242
INSTITUTIONS REPRESENTED—					
Total		57	80	77	79
By investigators	37	43	50	51	59
By students	31	36	41	47	42
SCHOOLS AND ACADEMIES REPRESENTED.					
By investigators	3	2	3	1	3
By students	9	1	6	5	9

4. SUBSCRIBING INSTITUTIONS—1915

AMHERST COLLEGE
BARNARD COLLEGE
BOWDOIN COLLEGE
BRYN MAWR COLLEGE
CARNEGIE INSTITUTE OF TECHNOLOGY
CARNEGIE INSTITUTION, WASHINGTON
CLARK UNIVERSITY
COLUMBIA UNIVERSITY
DARTMOUTH COLLEGE
ELSE SERINGHAUS SCHOLARSHIP, HUNTER COLLEGE, NEW YORK CITY
GOUCHER COLLEGE

HARVARD UNIVERSITY
JOHNS HOPKINS UNIVERSITY
KANSAS STATE AGRICULTURAL COLLEGE
LUCRETIA CROCKER SCHOLARSHIPS
MOUNT HOLYOKE COLLEGE
NORTHWESTERN UNIVERSITY
OBERLIN COLLEGE
PRINCETON UNIVERSITY, DEPT. OF BIOLOGY
PRINCETON UNIVERSITY, DEPT. OF PSYCHOLOGY
RADCLIFFE COLLEGE
ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
RUTGERS COLLEGE
SMITH COLLEGE
SYRACUSE UNIVERSITY
UNIVERSITY OF CHICAGO
UNIVERSITY OF ILLINOIS
UNIVERSITY OF KANSAS
UNIVERSITY OF MICHIGAN
UNIVERSITY OF PENNSYLVANIA
UNIVERSITY OF TEXAS
UNIVERSITY OF WISCONSIN
VASSAR COLLEGE
WELLESLEY COLLEGE
WESTERN COLLEGE
WISTAR INSTITUTE
YALE UNIVERSITY

5. EVENING LECTURES, 1915

Friday, July 2,
PROF. C. R. STOCKARD.. "Experimental Production of Racial Degeneracy by Alcohol Poisoning."

Tuesday, July 6,
PROF. C. H. PARKER.... "The Seals of the Pribiloff Islands."

Friday, July 9,
PROF. G. L. STREETER.. "Some Experimental Studies on the Development of the Membranous Labyrinth in the Tadpole."

Tuesday, July 13,
PROF. E. G. CONKLIN... "Effects of Centrifugal Force on the Structure and Development of the Egg."

Friday, July 16,
PROF. R. S. LILLIE....." The Nature of Intelligent and Purposive Action from a Physiological Point of View."
Monday, July 19,
PROF. SIMON FLEXNER.." The Control of Infection as Affected by Variation Among Parasitic Microörganisms."
Saturday, July 24,
PROF. G. N. CALKINS..." Protozoa and the Cancer Problem."
Friday, July 30,
PROF. GEORGE SHULL..." Inheritance of Sex in *Lychnis*."
Tuesday, Aug. 3,
PROF. C. B. DAVENPORT " Heredity of Criminality."
Friday, Aug. 6.
DR. MARTIN EDWARDS.." The Story of Bubonic Plague."
Tuesday, Aug. 10,
DR. ALFRED G. MAYER." The Rôle of Adsorption in Nerve Conduction."

6. MEMBERS OF THE CORPORATION

1. LIFE MEMBERS

ALLIS, MR. E. P., JR., Palais Carnoles, Menton, France.
ANDREWS, MRS. GWENDOLEN FOULKE, Baltimore, Md.
BILLINGS, MR. R. C., 66 Franklin St., Boston, Mass.
CAREY, MR. ARTHUR ASTOR, Fayerweather St., Boston, Mass.
CLARKE, PROF. S. F., Williams College, Williamstown, Mass.
CONKLIN, PROF. EDWIN G., Princeton University, Princeton, N. J.
CRANE, MR. C. R., Woods Hole, Mass.
DAVIS, MAJOR HENRY M., Syracuse, N. Y.
EVANS, MRS. GLENDOWER, 12 Otis Place, Boston, Mass.
FARLOW, PROF. W. G., Harvard University, Cambridge, Mass.
FAY, MISS S. B., 88 Mt. Vernon St., Boston, Mass.
FOLSOM, MISS AMY, 88 Marlboro St., Boston, Mass.
FOOT, MISS KATHERINE, 80 Madison Ave., New York City, N. Y.
GARDINER, MRS. E. G., Woods Hole, Mass.
GARDINER, MISS EUGENIA, 15 W. Cedar St., Boston, Mass.
HANNAMAN, MR. CHARLES E., 103 1st St., Troy, N. Y.

HARRISON, EX-PROVOST C. C., University of Pennsylvania, Philadelphia, Pa.
JACKSON, MISS M. C., 88 Marlboro St., Boston, Mass.
JACKSON, MR. CHAS. C., 24 Congress St., Boston, Mass.
KENNEDY, MR. GEO. G., 284 Warren St., Roxbury, Mass.
KIDDER, MR. C. G., 27 William St., New York City, N. Y.
KIDDER, MR. NATHANIEL T., Milton, Mass.
KING, MR. CHAS. A.
LEE, MRS. FREDERIC S., 279 Madison Ave., New York City, N. Y.
LOWELL, MR. A. LAWRENCE, 171 Marlboro St., Boston, Mass.
MARRS, MRS. LAURA NORCROSS, 9 Commonwealth Ave., Boston, Mass.
MASON, MR. E. F., 1 Walnut St., Boston, Mass.
MASON, MISS IDA M., 1 Walnut St. Boston, Mass.
MEANS, MR. JAMES HOWARD, 196 Beacon St., Boston, Mass.
MERRIMAN, MRS. DANIEL, Worcester, Mass.
MINNS, MISS SUSAN, 14 Louisburg Square, Boston, Mass.
MINNS, MR. THOMAS, 14 Louisburg Square, Boston, Mass.
MIXTER, MISS M. C., 241 Marlboro St., Boston, Mass.
MORGAN, MR. J. PIERPONT, JR., Wall and Broad Sts., New York City, N. Y.
MORGAN, PROF. T. H., Columbia University, New York City, N. Y.
MORGAN, MRS. T. H., New York City, N. Y.
NOYES, MISS EVA J., 28 South Willow St., Montclair, N. J.
NUNN, MR. LUCIAN L. Telluride, Colo.
OSBORN, PROF. HENRY F., American Museum of Natural History, New York.
PHILLIPS, DR. JOHN C., Windy Knob, Newham, Mass.
PHILLIPS, MRS. JOHN C., Windy Knob, Newham, Mass.
PORTER, DR. H. C., University of Pennsylvania, Philadelphia, Pa.
PULSIFER, MR. W. H., Newton Center, Mass.
ROGERS, MISS A. P., 5 Joy St., Boston, Mass.
SEARS, DR. HENRY F., 420 Beacon St., Boston, Mass.
SHEDD, MR. E. A.,
SMITH, MRS. C. C., 286 Marlboro St., Boston, Mass.
STROBELL, MISS E. C., 80 Madison Ave., New York City, N. Y.
THORNDIKE, DR. EDWARD L., Teachers College, Columbia University, New York City, N. Y.

TRELEASE, PROF. WILLIAM, University of Illinois, Champaign, Ill.
WARE, MISS MARY L., 41 Brimmer St., Boston, Mass.
WARREN, MRS. S. D., 67 Mt. Vernon St., Boston, Mass.
WHITNEY, MR. HENRY M., Brookline, Mass.
WILLCOX, MISS MARY A., Wellesley College, Wellesley, Mass.
WILMARTH, MRS. H. D., Elliott St., Jamaica Plain, Mass.
WILLIAMS, MRS. ANNA P., 505 Beacon St., Boston, Mass.
WILSON, DR. E. B., Columbia University, New York City, N. Y.
WILSON, PROF. W. P., Philadelphia Museum, Philadelphia, Pa.

2. MEMBERS, JANUARY, 1916

ABBOTT, PROF. J. F., Washington University, St. Louis, Mo.
ABBOTT, MISS MARGARET B., The Bennett School, Milbrook, N. Y.
ADDISON, DR. W. H. F., University of Pennsylvania, Medical School, Philadelphia, Pa.
ADKINS, MR. W. S., Texas Christian University, Fort Worth, Texas.
ALLEE, DR. W. C., Lake Forest College, Lake Forest, Ill.
ALLEN, PROF. EZRA, 125 Thompson Ave., Ardmore, Pa.
ALLYN, MISS HARRIET M., Hackett Medical College, Canton, China.
ALSBURG, DR. C. S., U. S. Dept. of Agriculture, Washington, D. C.
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AMITOSIS IN CELLS GROWING IN VITRO.

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(3 PLATES, 27 FIGURES.)

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INTRODUCTION.

During recent years the conviction among cytologists has become more and more strongly intrenched that the problem of amitosis can not be satisfactorily solved by investigations based alone upon the study of non-living tissue, but that its successful conquest must rely principally upon the correct interpretation of the succession of morphological and physiological changes revealed by prolonged observation of the living cell, under normal and artificially varied conditions. Yet until the advent of the tissue culture method, workers in biology might well have despaired of ever being able to attack the question in this way. It is fortunate, therefore, that the technique of tissue cultivation has been so perfected that even the minute structural details of the living cell may be readily observed, and that the inspection may be continued for hours at a time; fortunate, too, that configurations which may be interpreted as stages in the process of direct division, such as dumb-bell-shaped nuclei apparently undergoing constriction, and bipartite nuclei, are not infrequently found in tissue cultures, and, furthermore, that fixed and stained preparations, to assist in the interpretation of the appearances

presented by the living cells, are easily made from such cultures.

These favorable circumstances plainly indicated the course to be followed in an attempt to gain some knowledge of direct cell division, and accordingly continuous observations of living cells, and supplemental studies of fixed and stained cells of the same type, were carried out.[1]

Method

The cells upon which observations were made were growing at a temperature of 39° to 40° Cent. from the tissue of embryo chicks from two to ten days old. Cultures were prepared by the method of Lewis and Lewis ('15), Locke solution being used as a medium. Activation of the growth was accomplished by the addition to the medium of a small quantity of autogenous embryo extract or bouillon. Thus the medium contained, besides the various salts, small quantities of dextrose (.25 to 1 per cent.) and protein. To offset the concentration due to evaporation during planting and in the moist chamber it was found to be of advantage to dilute the medium by adding 20 to 25 per cent. of freshly distilled sterile water. Heart tissue was most frequently used, and gave growths which were most serviceable for observation during the second twenty-four hour period.

By arranging the microscope within the incubator where the tissues were cultivated it was not necessary to expose them to a changed temperature during observation. A ray screen of copper sulphate solution was found to be advantageous when artificial light was used. Evaporation of the drop, with condensation about the walls of the moist chamber, was lessened by placing a small drop of distilled water in the cavity of the depressed slide, and by eliminating air currents from the vicinity of the culture.

Light seemed to have a deleterious effect upon the living cultures, so that lengthy continuous observation was not found to be practicable. Accordingly inspections were made as short

[1] The procedure of studying amitosis by the tissue culture method was suggested by M. R. and W. H. Lewis, and I desire to record my appreciation of their kind assistance and the use of their large collection of fixed and stained cultures, which was utilized in the investigation. To Prof. F. R. Lillie I also am indebted for his courtesy in placing a room at the Marine Biological Laboratory at my disposal, where some of the work was carried on.

as possible, and the light immediately turned off. The difficulties attending direct and prolonged observation of the living cell are not inconsiderable, for the eye must become accustomed to distinguish minute structures through the contrast afforded by varying grades of refractivity. At first only the most refractive bodies are discernible, standing out as bright points or lines, but gradually the less obvious structures, as mitochondria, and amœboid cell processes, come into view. Migration, in many of the cells, is quite active, and hence it is necessary to make observations frequently to prevent the cell from wandering away from the field of vision and becoming lost. The extreme sensitivity of the cell to light and heat, and to changes in osmotic pressure of the media from evaporation within the moist chamber, is responsible for the untimely termination of many observations, and this difficulty becomes the more important when it is realized that the study of a single cell must cover many hours to be complete.

It was at first planned to select a single living normal cell and observe it at frequent intervals over a long period in the hope that eventually a cell would be found which would divide by amitosis. This course, however, did not prove to be practicable on account of the infrequency with which amitosis, even of the nucleus alone, is met with. In a series of 20 fixed and stained growths from the heart of the embryo chick, in which there was a total of 41,725 cells,[1] only 50 constricted nuclei, which could be regarded as directly dividing, were found (a ratio of 1 to 835), and thus the chances of the occurrence of amitotic nuclear division in a cell selected at random were but little better than one tenth of one per cent. To avoid loss of time, therefore, the plan was adopted of selecting a cell in which the amitotic process

[1] The method adopted in making counts was as follows: 20 good preparations from cultures of chick heart of various ages and stages of growth which had been fixed and stained were selected. A small square was ruled with a diamond upon a piece of glass after the method of Isaacs ('15), and this ruled glass was inserted in the ocular so that a definitely outlined field was marked off upon the tissue culture preparation on the stage. By manipulating the mechanical stage successive fields could easily be brought into view, and the cells contained in them counted; in this way all the cells in the entire new growth were counted except imperfect cells and those near the original piece, which were several layers deep and were indistinct.

appeared to have already commenced, viz., in which the nucleus showed elongation and equatorial constriction, and observing in detail the subsequent changes which it underwent.

Such a cell presents an appearance of the following general type. The nucleus is somewhat lengthened, and, in the zone equidistant from its poles there is to be seen, on one or both sides, an indentation. In this concavity is situated characteristically a body, the centrosphere (fig. 1, *c*) whose refractivity is somewhat greater than that of the surrounding cytoplasm. Its outline is indistinct, but the edge seems to be irregular with short toothlike processes. These change their shape slowly, and give the impression of being pushed out and drawn in very gradually. They are intimately related to definite refractive bodies—the mitochondria—which, often rodlike and sometimes threadlike in form, radiate from the periphery of the centrosphere. Indeed the movements of the latter (described by Lewis and Lewis '15) may be responsible for the apparent movement in the periphery of the centrosphere.

Preparations fixed with osmic acid vapor and stained with Heidenhain's iron hematoxylin (which was the method generally employed) disclosed a minute granular body, generally paired, within the centrosphere, which is recognized as the centrosome or centriole (Fig. 24).

The position of the centrosphere within the nuclear concavity or cleft has been noted by various authors, including Maximow ('08), who found the centriole-pair thus situated in cells, the nuclei of which appeared to be dividing directly, in the mesenchyme of the embryo rabbit. In the cells of tissue cultures examined the centrosphere was absent from the cleft in only two per cent. of cases, and in these exceptions it may have been originally situated as in the others.

Observations.

A number of extended observations were made upon living cells containing such elongated and constricted nuclei, and, as a general rule, the nucleus rounded out again, assuming the usual form. Thus it was demonstrated that constriction alone does not indicate that the nucleus will divide directly. Finally,

however, a cell was found in which the nucleus divided directly while being watched, and the following paragraph, extracted from the protocol written at the time, is a brief description of the process as observed. The drawings (Plate I.) were sketched free-hand from observation at fifteen-minute intervals, and afterward retouched by reference to fixed and stained cells of similar morphology.

8.45 P.M. A cell (Fig. 1), growing from a 57-hour culture of 5-day chick heart, presented an elongated nucleus with a concavity upon one side. The outline of this concavity was indistinct on account of the fact that the centrosphere (*c*) was situated very close to it.

9.00 P.M. (Fig. 2). The nucleus is now straight and the indentation is almost obliterated.

9.15 P.M. (Fig. 3). The general outline of the cell has changed, and this has been followed by change in shape of the nucleus. At first there were two nucleoli within the nucleus, the lowermost being paired, but at 9.30 (Fig. 4) the latter appears as a dumb-bell-shaped body. The nucleus is now rounded.

9.45 P.M. (Fig. 5). The nucleus is still rounded, and the nucleolar substance consists of two masses close together.

10.00 P.M. (Fig. 6). The nucleus has become elongated again. There is no apparent cleft, but the left side, against which the centrosphere is resting is not so distinct as the right. The central nucleolus now appears as a single structure and some refractive substance, resembling another nucleolus, is seen in the lowermost pole of the nucleus.

During the next two fifteen-minute intervals (Figs. 7 and 8) a shortening of the nucleus occurred, a shallow concavity to the left being noted. This concavity is deeper at 10.45 (Fig. 9) its outlines being somewhat indistinct and the nucleus has increased in length; across the middle of the nucleus is a refractive line.

11.00 P.M. (Fig. 10). The concavity is seen with difficulty, and the line across the nucleus persists. Fifteen minutes after this (Fig. 11) the elongated nucleus shows an indentation upon the right side, opposite the one upon the left, and it appears to be undergoing constriction into two parts of equal size. Between the two nuclear portions the refractive line is seen as before, and, from the appearance shown in cells fixed and stained with iron hematoxylin (Fig. 24) this is evidently a strand of mitochondria.

11.30 P.M. (Fig. 12). The nuclear sacs are apparently quite separate, and between them is the strand of mitochondria, and also part of the centrosphere, this body, still undivided, having retained its position with reference to the nuclear cleft.

Judging from the behavior of this and other elongated, bent and dumb-bell shaped nuclei it would seem that the nucleus may return to its original rounded form provided the constriction has not gone too far, but, if the degree of constriction passes a certain critical point the nuclear sacs become completely separated.

Furthermore, after the critical point is passed the division occurs very rapidly.

The study of fixed and stained specimens served to throw considerable light upon the process of direct division, for, by searching the field, transitional forms were encountered, suggesting stages in the history of the living nucleus just described. In the terminal stage of direct nuclear division, such as that shown in Fig. 24, mitochondria were found characteristically lying across the slender strand of nuclear membrane which was all that remained of the connection between the two portions of the nucleus, and the centrosphere (which is undivided in these transitional forms, and also in the end product of nuclear amitosis, the binucleate cells) is situated in the cleft dividing these parts.

This process involves a more or less equal mass division of the nucleus without chromosome formation. It results in the production of a binucleate cell, and, if the process is repeated, of a trinucleate or multinucleate cell. Direct nuclear division was not observed beyond the bipartite stage, but it seems rational to suppose that (excluding the foreign body giant cells of Lambert (1912 *a* and *b*) the giant cells of tissue cultures are formed from the successive direct splitting of the nuclear fragments which become larger by normal growth. With this latter there is apparently associated an increase in the cytoplasm also.

It was not practicable to make a complete study of amitosis upon the same cell, and the latter stages of this process, succeeding direct division of the nucleus, were studied by selecting living binucleate cells and making prolonged observations upon them. A typical case, from a 24-hour culture of 8-day chick heart, is given as follows:

11.50 A.M. The cell, which was of the characteristic connective tissue type, showed two separate nuclear sacs, whose adjacent surfaces were in close contact. One sac contained three nucleolar fragments, the other one. Fat granules were fairly abundant, and were principally congregated at the nuclear poles. Mitochondria, long and threadlike, stretched between these granules and, where the latter were abundant, the strands of mitochondria tended to arrange them in rows. Mitochondria also radiated from the single centrosphere, situated opposite the area of contact of the two nuclear sacs. The triangularly shaped cell body was connected with adjacent cells by three main processes.

12.20 P.M. A narrow interval can be seen between the nuclear parts, showing that the latter have moved apart and are quite separate. Their position also has

changed. Variation in the nucleoli is noted, there being now three in one nuclear sac and four in the other. The outline of the cell body is now quadrilateral, and this shifting of form has perhaps accounted for the rearrangement in relative position and relationship of the nuclear parts. These, at 2.30, were again in contact, but subsequently repeated the process of moving apart and coming together three times during the observation, which ended at 11.15 p.m. During this time (11½ hours) the cell was observed continuously, and underwent constant minor changes, such as that of the outline, shifting about of cytoplasmic structures, and breaking up, recombination and variation in size and shape of the nucleolar fragments. After almost twelve hours the cytoplasm showed no indication of dividing.

These observations brought out the fact that what might be mistaken for a single nucleus divided by a membrane across its equator is really two nuclear sacs pressed close together, the equal tension in the two bodies resulting in a flat membrane between them, made up of the surfaces of contact. Child (1911) describes a type of amitosis which is characterized apparently by the growth through the nucleus of a membrane or plate, the subsequent splitting of which leads to the production of two nuclear sacs quite separate one from the other. Nuclear fission of this type was not found, and appearances suggesting a process of this kind probably result from the close relationship of separate nuclear parts, similar to the condition found in the cells of tissue cultures. Partitions within the nucleus are also simulated, in these flattened cells, by long nucleoli, mitochondria or folds in the nuclear membrane.

These observations also illustrate the characteristic behavior of the nucleolar bodies, which undergo constant changes in size, shape, number and position. These bodies stain well with gentian violet when applied to the living culture. They appear as dark masses after iron hematoxylin, but if differentiation with iron alum is carried too far what was before a homogeneous mass becomes a collection of granules (Fig. 27). The nucleolus appears to be a concentrated gel of varying density, the granules representing the denser areas.

Similar observations upon other living cells were carried out, and in no case did any direct fission of the cytoplasm occur; this finding was supported by the study of fixed preparations. On the contrary the history of these binucleate cells was the same as that of mononucleate cells of the same type. It was even found that mitosis occurred in these cells, for, during the obser-

vation of a binucleate cell in a young culture the good fortune was encountered of witnessing this entire process in it. A graphic register of the successive changes is afforded by the drawings (Plate II.) which were made with the aid of a camera lucida at the times stated. The following is extracted from the protocol written at the time of examination:

11.55 A.M. A typical connective tissue binucleate cell (Fig. 13) from a 19-hour culture of 7-day chick heart was selected for observation. The two approximately equal, sharply outlined nuclear sacs are in close contact, causing a flattening of the apposed surfaces. Each nucleus contains a single, somewhat irregular nucleolus. The cell is long and narrow, and the long axis of the nucleus is parallel with that of the cell in which it is contained. A single centrosphere (*c*) is found opposite the area of contact of the two nuclear parts. Numerous fat globules and mitochondria, the latter showing characteristic movements, are seen.

12.40 P.M. (Fig. 14). The nucleus is still double, and the principal change noted is the appearance of an additional nucleolus in the lower nuclear part.

1.20 P.M. (Fig. 15). The two parts of the nucleus are distinct. Only one nucleolus is now seen in each nuclear sac.

1.50 P.M. (Fig. 16). The cell outline has become modified, the cell body being shorter, and the long axis of the nucleus has changed so that it is now almost at right angles to its former direction. The nuclear surfaces are in close contact and the upper end of the membrane formed by their approximation is indefinite in outline.

3.05 P.M. (Fig. 17). The cell body has become more fusiform, and the long axis of the nucleus has rotated through 90°. The double membrane (formed by the areas of nuclear wall in contact) dividing the nuclear portions cannot be made out except at the left side, and there indistinctly. An indefinite, refractive substance, granular in character, is scattered along the line where this membrane had been; the nucleoli are indefinite, and the uppermost one has been joined by an additional, very small, mass of the same character. The upper part of the nuclear membrane is not so distinct as heretofore.

It would appear that the change which has occurred in the part of the nuclear membrane dividing the two nuclear sacs is a part of the general change affecting the entire nuclear wall and leading to its gradual disappearance, and the process is similar to that which occurs in the early stages of mitosis.

5.05 P.M. (Fig. 18). The refractive material in the zone formerly separating the two nuclear sacs is more prominent than before; it seems to be chromatin. The nuclear membrane is faintly marked.

Half an hour later this cell is seen to become gradually smaller, and to draw in its processes. At the same time the nuclear parts become smaller. The nucleoli also undergo diminution in size. Finally the cell takes a rounded and thickened form—6.00 P.M. (Fig. 19)—and is much more refractive than the cells surrounding it: in fact it resembles a cell in the prophase of mitosis. The fat globules and mitochondria assume a wreath-like appearance about the central clear space, in which the nucleoli soon disappear. Though the main mass of the cell is almost circular there are narrow processes attached to each pole. The cell remains apparently unchanged for some time, though undoubtedly important readjustments are going on within it.

6.50 P.M. (Fig. 20). The cell is even smaller than before, and more rounded. In the clear area within it is a refractive bar, which proves to be the equatorial plate of chromosomes. Individual chromosomes cannot be distinguished, so that it is impossible to count them, but their ends may be seen, as they project towards the poles of the spindle. The chromosomes show a slow, oscillatory type of movement, slight in extent. The spindle is represented by a fairly clear area, shaped like two cones base to base, at the extremities of which the centrosomes are situated. Astral rays cannot be seen. Mitochondria and fat globules encase this central area containing the spindle like a shell. This becomes evident by focusing up and down. Though the uppermost part of the spherical cell is on a level with the flat resting cells, which are to be found about it, the lowermost part is much below this, due to the fact that the cell is thickened, and projects into the medium.

7.05 P.M. (Fig. 21). The cell has suddenly become elongated and constricted at the equator; the plate of chromosomes has evidently split, and the anaphase of mitosis is being witnessed. The constriction about the middle of the cell can be seen to be increasing, causing streaming of globules toward its extremities. At the same time small, bubble-like processes emerge from the borders of the cell, seeming to be forced out by the internal pressure of the cell body. Into these protuberances granules flow but later return into the main mass of the cytoplasm. These processes soon become flattened and extended, forming pseudopodia possessed of hyaline borders with amœboid movement. The individual chromosomes of the two masses in the expanded extremities gradually lose their distinctness and become dispersed.

7.25 P.M. (Fig. 22). The constriction is more marked and the cells are almost entirely separated. They are also becoming flattened out. In the upper daughter cell a clear space for the nucleus is appearing. The constricted zone is somewhat more highly refractive than the surrounding tissue and resembles a short thread. This probably contains part of the remains of the spindle. Nuclear details are not yet visible.

As the cell is watched nucleoli become manifest, at 7.35 P.M. two of these being seen in each daughter nucleus in a clear space, surrounded by a distinct nuclear membrane.

8.00 P.M. (Fig. 23). The daughter cells are now almost of normal size. Each nucleus has a concave side, as is usual, and in each concavity is the new centrosphere, from which the mitochondria radiate. Two nucleoli appear in each nucleus. The cells are spread out and flattened, and the fat granules are disposed as in the ordinary cell.

The entire observation covered eight hours. The more active part of mitotic division occupied about two hours, but if the initial nuclear changes be included the duration of mitosis is much longer.

The sequence of changes followed in the above cell are in almost all respects similar to those of mitosis many times observed in the mononucleate cell. The only difference is that in the cell described there were to begin with two separate nuclear sacs instead of one. From the presence, in fixed preparations, of bipartite nuclei each portion of which is in a condition of spireme, and the absence, from such preparations, of bipartite nuclei in

which one portion only contains a skein, it is gathered that the prophase in these cells is characterized by the spireme appearing coincidently in the separate nuclear sacs (Fig. 25); later there is formed from the double spireme a single equatorial plate of chromosomes. Though these could not be counted there is no reason for believing that their number was more than that normal for the mononucleate cell.

Not only has mitosis been demonstrated by observation, both on living and fixed preparations, to occur in binucleate cells, but it has been found that it occurs relatively as frequently in these cells as in those with a unipartite nucleus. By making cell counts in the aforementioned 20 preparations from chick heart 375 binucleate cells were found in a total cell count of 41,725. Thus binucleate cells made up a percentage of the total of 0.9, or a ratio of 1 in 111. Among these binucleate cells 2 were found which were in the prophase of mitosis,[1] a percentage of 0.53. Of the mononucleate cells, which numbered 41,106, 47 were in the prophase, or 0.114 per cent. By comparison of these ratios it is found that mitosis occurs 4.65 times as frequently among the binucleate as among the mononucleate cells; allowing for the limited scope of the observation it seems reasonable to conclude that mitosis is as frequent a phase of the life of binucleate cells as of mononucleate, and it would seem that this is their normal method of proliferation. If, in addition, they be considered as dividing by direct fission (for which there is no evidence either from living or fixed material) they would then be possessed of an ability to multiply in excess of that of the mononucleate cells, and there is no reason for supposing this to be the case.

No evidence was brought to light that the separate parts of the bipartite or multipartite nucleus ever combine except during mitosis.

Another interesting observation was made in a fixed preparation, viz., that the early changes in the chromatin which presage the onset of mitosis (*i. e.*, the clumping of the chromatin and its

[1] Prophases alone were counted, in estimating cells in mitosis, since in this stage alone is it possible to distinguish the bipartite from the monopartite nucleus, on account of nuclear fusion in stages later than this in the case of mitosis in the binucleate cell.

segregation into short rodlike masses and finally into a spireme) can take place in a nucleus which is undergoing constriction. Thus it appears that mitosis can proceed as usual in nuclei partially divided or wholly divided by the amitotic process.

Discussion.

The direct division of the nucleus is associated with certain changes of the cell as a whole. Elongation of the nucleus seems to be a prerequisite, and this apparently is secondary to a lengthening and narrowing of the cell body occasioned by the pull of its processes. It has been pointed out that the centrosphere is situated characteristically in a concavity at one side of the nucleus, and, when the nucleus lengthens, this body sinks deeper into its side; at the same time, judging from fixed preparations, and also from the appearance of the living and dividing nucleus, mitochondria come to lie across this narrow nuclear isthmus. These bodies, the centrosphere and associated mitochondria, seem to play a part in the fission of the nucleus. The exact manner of their action is not clear, but it may be that streaming of the nucleoplasm away from the equator of the nucleus follows upon the mechanical irritation of the nuclear membrane by their movements, or possibly upon local alteration of surface tension from their chemical change. Certainly the position which these bodies take with reference to the constricted nucleus points to their participation in the process of fission.

The centrosphere does not divide, nor does it encircle the nucleus as in the form of division described by Meves ('91). The nuclear membrane remains intact and nothing resembling an amphiaster is formed.

Some theories of amitosis have attempted to place the responsibility for the initiation of the divisional stimulus upon the nucleolus, and it has been found by some investigators that in certain nuclei the nucleolus is the first body to become divided. Such a function of the nucleolus does not obtain, however, in the nuclei studied, for, although in some cases there was a division of the nucleolus into two parts, one of which became alloted to each separate nuclear part, yet, sometimes the nucleolus did not divide, and one of the nuclear fragments was without visible

nucleolar material (Fig. 24). Occasionally two fragments would be found in one nuclear part while the other had none; again it was very frequently found—indeed it was the rule—that a nucleus would have two separate nucleolar fragments without any nuclear division. In short not only was the division of the nucleolus in most cases not followed by nuclear fission, but the latter took place in many cases without division of the nucleolus.

Neither before nor after nuclear fission was there discovered an instance of differential staining of the two halves of a nucleus about to be divided, or already divided, as shown by Child ('07) so that there was no evidence of this kind to support the belief of Child that there may be a physiological independence of the nuclear areas even before they become amitotically divided, manifested by a variation in the staining of the two nuclear halves. The study of living nuclei, too, divulged nothing in favor of this hypothesis.

The result of this process of nuclear splitting was the formation of a cell containing one or more separate nuclei of about equal size, and each of about the same size as the nuclei of mononucleate cells. After nuclear fission the separated nuclear elements manifested the power of growth, and seemed to have metabolic independence. The cell protoplasm also of these cells shows an ability to increase in bulk. This is especially evident in giant cells which can thus become quite large.

It is believed, furthermore, that binucleate cells and giant cells in tissue cultures (except foreign body giant cells which arise by fusion of previously separate cells) do not arise in any other manner than that above outlined, for there is no other adequate explanation of their origin. A careful examination of living and fixed material does not reveal any evidence of fusion of cytoplasm without fusion of the nuclei, so that there are no grounds for admitting this as a possible theory of formation. Although many of the binucleate cells undoubtedly do migrate as such from the original piece, where they are doubtless formed in the same manner as they are in the new growth, yet an increase of over 100 per cent. in their number in the growth of the second day as compared with that of the first (as ascertained by making careful counts of the 20 heart specimens aforementioned) can

hardly be explained by the assumption of an increased emigration of these forms during the second day; it is more probable that some of these bipartite nuclei have originated in the new growth, and the observation of this process here confirms this conclusion.

Mitosis, too, cannot account for the formation of these bi- and multipartite nuclei, for in all the cases of mitosis followed through in the living condition the end result has always been two daughter cells, quite separate except for a narrow connecting process, and each containing a single centrosphere. The only theories remaining for consideration are those of nuclear origin *de novo*, or from chromidial extrusions, and these suppositions are too improbable to discuss here. No other process than that of nuclear amitosis, therefore, can account for the production of bi- and multinucleate cells in the cultures examined.

Though the amitotically-divided parts of the nucleus seem to possess metabolic independence, as noted, they do not appear to have reproductive independence, for the reason that they are never dissociated one from the other to become the nuclei of separate cells. Furthermore they show only one type of cell division, viz., mitosis, in which the process begins coincidently in the two nuclear parts, manifested by the simultaneous appearance in each part of a similar spireme. Although in amitosis there is a mass division of the nuclear material there is no meristic division, and it appears that before a cell containing an amitotically divided nucleus can divide it is necessary that the separated chromatin moieties should recombine. This was done in the specimen examined during life, for the combined product of the two nuclear sacs formed a single equatorial plate of chromosomes. Such a type of amitosis, therefore, is not incompatible with the chromosome hypothesis.

Spiremes in bipartite nuclei, and in dumb-bell-shaped nuclei evidently undergoing amitosis, are not confined to the cells of tissue cultures, for Maximow ('08) has described them in the normally-developing mesenchyme cells of the embryo rabbit, and this author refers to similar cell configurations which Karpow ('04) describes in the leucocytes of urodele amphibia.

Fragmentation.

A note may here be made regarding a pathological change which nuclei subjected to unfavorable conditions undergo, viz., fragmentation. This change consists in a breaking up and degeneration of the nuclei. In cells which had grown for six days in unchanged media, in which food and oxygen had become depleted and metabolic products had accumulated (Fig. 26) and also in cells growing in a medium to which a small amount of ethyl alcohol had been added (Fig. 27), this form of degeneration was seen. Multilobulation of the nucleus appeared to antecede the actual breaking away of the parts. These latter were of different shapes and sizes, often did not contain a nucleolus, and showed no power of growth. The cytoplasm enveloping them did not divide and apparently was incapable of increasing.

In preparations containing forms of this kind no mitoses were seen, and the phenomenon seemed to be quite different from nuclear amitosis which occurred only in healthy cells. It is believed, moreover, that the process of fragmentation has been confused with that of amitosis, and it is possible that it is this confusion which has accounted for certain well-known views which regard amitosis as an evidence of degeneration.

Vital Stains.

Finally, a word as to certain so-called "vital stains." It was hoped that gentian violet would prove to be of value in rendering visible the *minutiæ* of the living cell during its vital changes, since the results following the use of this dye recorded by Churchman and Russell ('14) and Russell ('14) with cultures of frog tissue were so encouraging. Unfortunately the dye proved toxic to the tissues used in a dilution of 1 in 200,000, and, though the nucleoli, nuclear membranes, certain granules and the cell borders were brought into sharp relief this staining was always accompanied by cessation of vital phenomena, and the cells speedily went into degeneration.

Janus green, in a dilution of 1 in 80,000, was also toxic, and, while it stained mitochondria specifically, yet these bodies soon became granular and lost their characteristic appearance. Hence neither of these dyes could be spoken of as acting "*intravitam*,"

and they were of value only in obtaining rapidly information as to the obscure structure of the cell; the cell, however, was thus sacrificed.

SUMMARY.

The observations above described and the interpretations thereof may be briefly summarized as follows:

Amitosis was found to involve only the nucleus and was not a method of cell proliferation. It occurred in normal cells and was characterized by a separation of the nucleus into one or more parts which possessed no reproductive independence.

The process of nuclear amitosis consisted in a unilateral or bilateral constriction, manifested by a narrowing of the nucleus in the region of its equator, and a streaming of its contents toward the poles, with final separation of the two nuclear portions. This phenomenon seemed to be associated with the action of the mitochondria and centrosphere upon an elongated nucleus. There was no amphiaster or spireme formation and no centrosome fission. Division of the nucleolus was not an essential. Repetition of this process leads to the formation of a giant cell.

Not all nuclei which show elongation and constriction divide by direct fission, but many return to their usual rounded or oval form. When, however, the constriction has passed a critical point the division goes on to completion, and this final stage is rapid.

Cells containing nuclei in process of, or the result of, amitosis divide by mitosis. Mitosis in binucleate cells, which are the product of nuclear amitosis, is characterized by the simultaneous appearance in the nuclear parts of a spireme, from which a single equatorial plate of chromosomes is formed. Furthermore, binucleate cells divide as frequently by mitosis as do mononucleate cells, and this was the only form of division found to occur in them.

Since the parts of an amitotically divided nucleus do not become separated as reproductive units but divide only by mitosis, in which the chromatin in the parts is recombined, there is nothing in nuclear amitosis opposed to the chromosome hypothesis.

The type of amitosis in which the nucleus is split by the growth

through it from one side to the other of a membrane was not found. Nuclear figures simulating this proved to be caused by the close apposition of separate nuclear sacs, or by nucleoli, mitochondria or folds of the nuclear membrane.

The dyes, janus green and gentian violet, were toxic and their presence in the cell was incompatible with its continued life. They were, however, of service in quickly studying structural details which were not discernible in the living state.

Nuclear fragmentation, which differs in many ways from nuclear amitosis, is a pathological condition, and occurs in degenerating cultures.

It is believed that the facts brought to light through the tissue culture method may be applied to the interpretation of the phenomena of normally developing cells.

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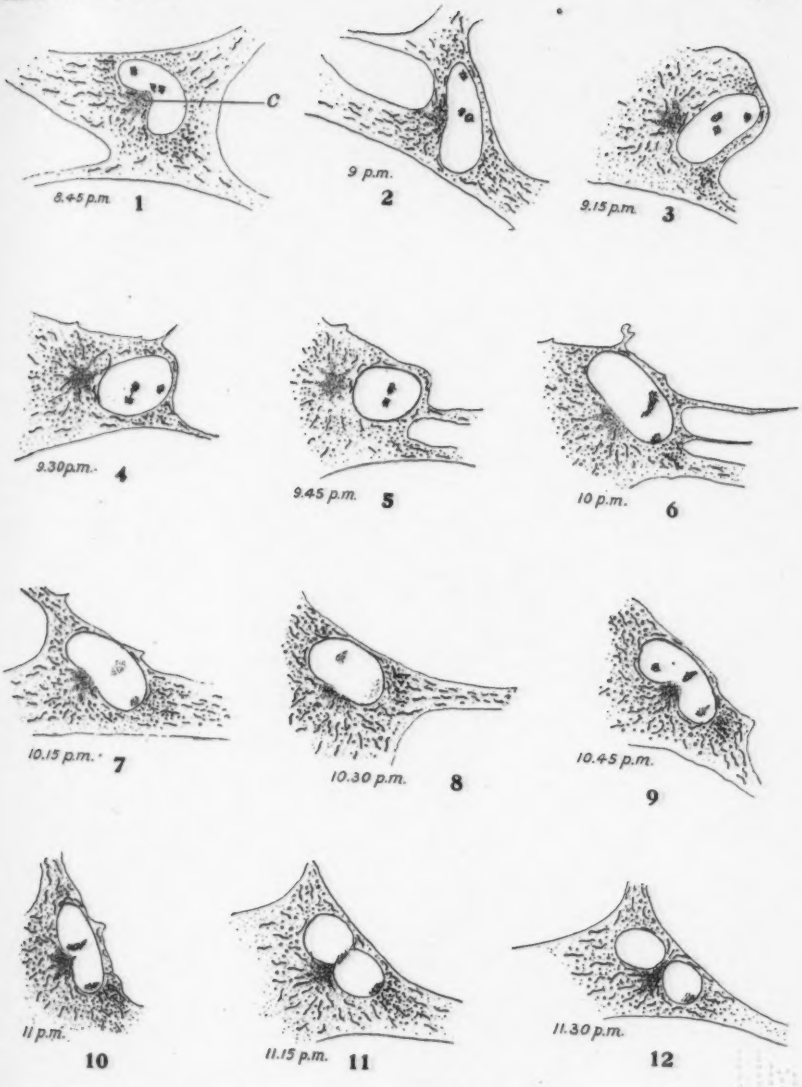
EXPLANATION OF PLATES.

PLATE I.

FIGS. 1 TO 12. Successive stages covering a period of 2¾ hours in the life history of a connective tissue cell from a 57-hour culture of 5-day chick heart, growing in Locke solution (0.5 per cent. dextrose with extract of chick embryo). The nucleus finally divides by direct fission. Small circles represent fat globules and short threads mitochondria. *c*, Fig. 1, points to the centrosphere. Free-hand drawings × about 900. (Description in text.)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8.45 p.m. 1
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9.15 p.m. 3
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10.30 p.m. 8
10.45 p.m. 9
11 p.m. 10
11.15 p.m. 11
11.30 p.m. 12

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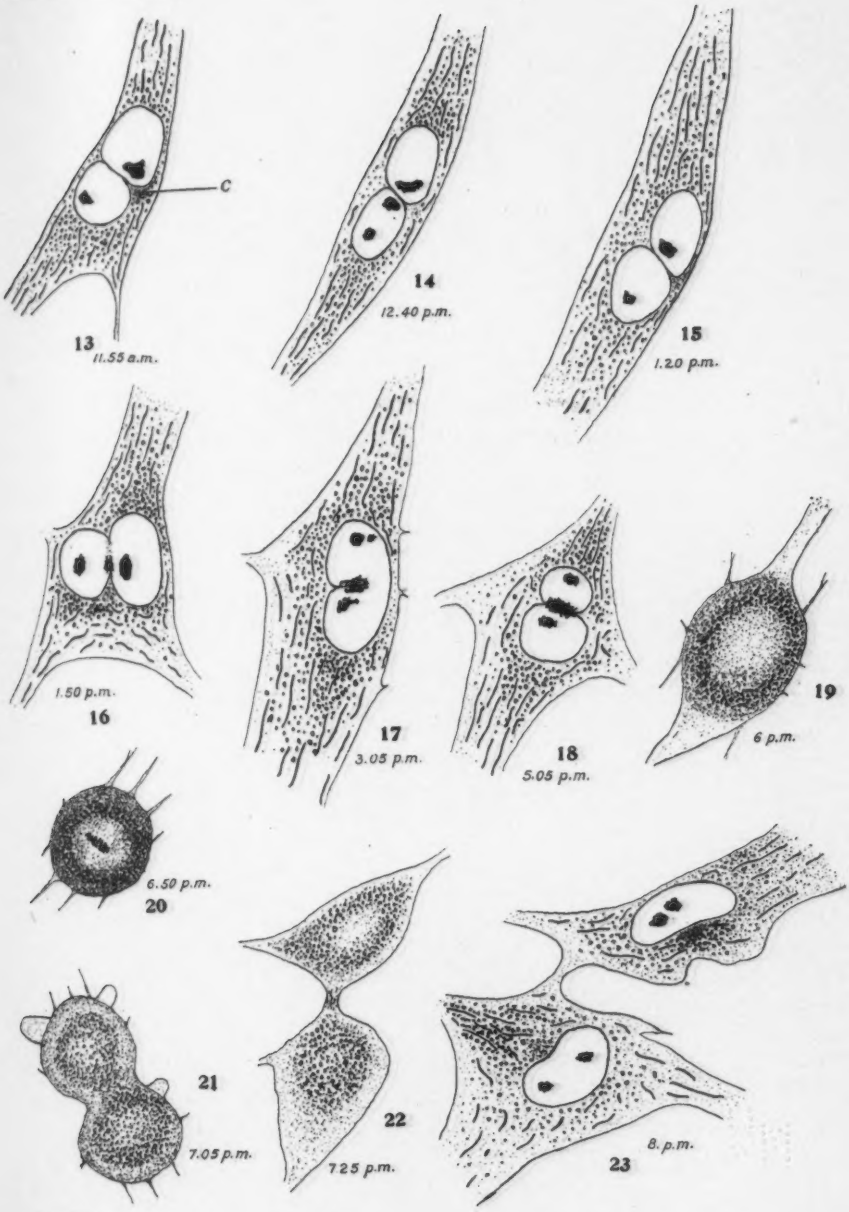
PLATE II.

FIGS. 13 TO 23. Graphic record of the process of mitosis in a living binucleate cell, from a 19-hour culture of 7-day chick heart, growing in Locke solution (1 per cent. dextrose with extract of chick embryo). *c*, Fig. 13, points to the centrosphere. Camera lucida drawings. × 1,333. (Description in text.)


C
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8. p.m.
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PLATE III.

FIG. 24. Direct nuclear division in a connective-tissue cell, final stage. Centrosphere between the nuclear parts. Across the slender filament joining these is a strand of mitochondria. Nucleolus has not divided. Camera lucida drawing from Preparation No. 2, from 5-day culture of 7-day chick heart in Locke solution (1 per cent. dextrose); osmic acid vapor and iron hematoxylin. × 915.

FIG. 25. Spireme in a bipartite nucleus. Prophase of mitosis. Nuclear membrane and nucleoli are disappearing. Camera lucida drawing from Prep. No. 14, 9-1-15 (Lewis collection). Heart from 6-day chick grown in Locke (0.5 per cent. dextrose) with a little yolk; fixed on third day of growth in Zenker; stained with iron hematoxylin (this culture was originally stained with Mallory's connective tissue stain). On account of the method of fixation the cytoplasmic details are not shown. × 915.

FIG. 26. Nuclei showing fragmentation. Camera lucida drawings from Prep. No. 23, 12-1-15 (Lewis collection). 5-day chick stomach in Locke (0.5 per cent. dextrose). Zenker; Mallory connective tissue stain. Culture grown for 6 days in the same media. × 1,012.

FIG. 27. Nuclei showing fragmentation. Camera lucida drawings from Prep. No. 23, 24-11-14 (Lewis collection). 6-day chick stomach in Locke (1 per cent. dextrose) to which ethyl alcohol had been added to make approximately 1 per cent. 3-day culture. Osmic acid vapor and iron hematoxylin. × 1,500.

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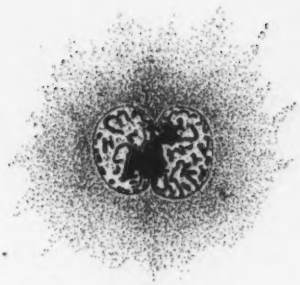

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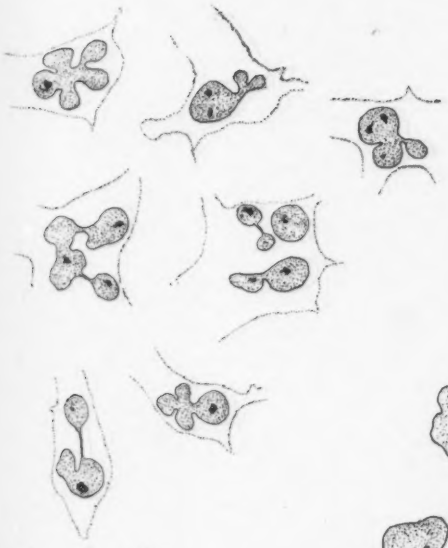

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